Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: M. S. Afifi (2003) A Novel 4'-*O*-Diglycoside of Decarboxyrosmarinic Acid from *Blepharis ciliaris*, Pharmaceutical Biology, 41:7, 487-490, DOI: 10.1080/13880200308951340

To link to this article: https://doi.org/10.1080/13880200308951340

Pharmaceutical Biology
2003, Vol. 41, No. 7, pp. 487–490

Taylor & Francis
Taylor & Francis Group

A Novel 4′-*O*-Diglycoside of Decarboxyrosmarinic Acid from *Blepharis ciliaris*

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Abstract

A novel natural phenolic **1** was isolated from the hydroalcoholic extract of the aerial parts of *Blepharis ciliaris* (L.) B.L. Burtt (Acanthaceae), in addition to apigenin 7-*O*-glucoside **2** and apigenin-7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl glucoside) **3**. The structure of **1** was established as 3′,4′-dihydroxy-β-phenyl ethyl caffeate-4′-β-*O*-D-galactopyranosyl-(1‴→4″)-α-*O*-L-rhamnopyranoside [= 9′-decarboxy rosmarinic acid-4′-*O*-(1→4)-galactosyl rhamnoside]. Structures were determined by conventional methods of analysis, as well as by different MS and NMR techniques.

Keywords: *Blepharis ciliaris*, Acanthaceae, phenolics, flavonoids, 9′-decarboxy-rosmarinic acid-4′-*O*-(1→4)-galactosyl rhamnoside.

Introduction

The family Acanthaceae comprises 346 genera and 4,300 species distributed in tropical and temperate regions (Ghazanfar, 1994). Certain species are used in traditional medicine in Somalia (Samuelsson et al., 1991), Tanzania (Chhabra et al., 1987) and Saudi Arabia (Ageel et al., 1987). Certain members of the family were shown to contain alkaloids (Ghazanfar, 1994; Adesomoju, 1982; Youhnovski et al., 1999), lignans (Hussein Ayoub, 1987), tannins, cyanogenic glycosides and saponins (Ghazanfar, 1994; Rizk et al., 1985). Anti-inflammatory (Mruthyunjayaswamy et al., 1998) and antimicrobial (Meurer-Grimes et al., 1996) activities have been demonstrated for extracts of certain plants belonging to this family. A member of the Acanthaceae, namely, *Blepharis ciliaris* (L.) B.L. Burtt is distributed throughout tropical Africa, Egypt, Arabia and Pakistan (Ghazanfar, 1994; Ageel et al., 1987; Tackholm, 1974). In spite of the use of the plant as a fodder, the seeds are used in Pakistan as a diuretic and aphrodisiac (Ghazanfar, 1994). The calyx and capsule including seeds is used as disinfectant and haemostatic for wounds and cuts. In addition, charcoal from the roots is applied to the eyes to improve vision, hence, the Arabic name "Kohl-el-agouz" (Batanouny et al., 1999). A recent pharmacological study of the hydroalcoholic extract of *B. ciliaris* growing in Egypt, carried out in co-ordination with the present work, revealed significant hepatoprotective, antihepatotoxic, anti-inflammatory, analgesic, diuretic and haemostatic activities (Zakaria, 2001). 7-*O*-6″-*p*-Coumaroyl glucosides of both apigenin and naringenin were reported from the seeds of *B. sindica* (Ahmed et al., 1984), while the respective 3″-acetyl congeners were reported from *B. ciliaris* (Harraz et al., 1996). This paper reports the isolation and structure elucidation of the novel 9′-decarboxy rosmarinic acid-4′-*O*-(1→4)-galactosyl rhamnoside **1** from the aerial parts of *B. ciliaris* collected in Egypt, in addition to apigenin-7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl glucoside) **2** and apigenin-7-*O*-glucoside **3** which were found for the first time in this plant.

Materials and methods

Plant material

The aerial parts of *Blepharis ciliaris* (L.) B.L. Burtt [Syn: *Ruella ciliaris* L.; *Blepharis edulis* (Forssk.); *Blepharis persica* (Burm.f.) Kuntze] were collected before flowering in October 1998 from Wadi Hasana, South Sinai, Egypt, and authenticated by Dr. M.El-Gibaly, Taxonomist, Dept. of Chemistry of Natural and Microbial Products, where a voucher specimen (B-10/1998) is kept.

Accepted: May 15, 2003

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1388-0209/03/4107-487$22.00

Apparatus and methods

NMR: Jeol EX-270 spectrometer, 270 MHz (^{1}H-NMR) and 67.5 MHz (^{13}C-NMR), respectively. ^{1}H Resonances were measured relative to TMS and ^{13}C-NMR Resonances to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width, 5000 Hz for ^{1}H and 2000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. Mass spectra were recorded using a Micromass Quatro-LC triple quadrupole mass spectrometer equipped with a *Z*-spray electrospray ion source; capillary voltage, 3 KV; source block temperature, to 120 °C; cone gas, 60 L/h; desolvation gas, 520 L/h.; desolvation temperature, to 150 °C; scan-range, *m/z* 50–1500. UV spectra were run in MeOH, using a Shimadzu UV-240 spectrophotometer. PC (descending): Whatman No. 1 sheets, using solvent systems: (1) H_2O (2) 15% acetic acid (3) BAW (*n*-buOH-HOAc-H_2O, 4 : 1 : 5, upper layer) (4) C_6H_6-*n*-BuOH-H_2O-pyridine (1 : 5 : 3 : 3, upper layer). Solvent systems 3 and 4 were used for sugar analysis.

Extraction, phytochemical screening, isolation and characterization

The powdered air-dried aerial parts (2.5 kg) were exhaustively extracted by refluxing with EtOH : H_2O (3 : 1, v/v) on a boiling water bath (3 × 4 L, each for 8 h). A phytochemical screening (Farnsworth, 1966) of the extract revealed the presence of phenolics, alkaloids and saponins. After stripping-off the solvent, the extract was subjected to CC on polyamide 65 (Riedel-De Haen AG, Germany) and eluted with H_2O followed by H_2O-EtOH mixtures of decreasing polarities to yield nine fractions, which were individually subjected to 2D-PC. The phenolic fraction eluted with H_2O-EtOH mixture (80 : 20) was subjected to repeated column fractionation on Sephadex LH-20 (Pharmacia, Sweden) using water-saturated-*n*-BuOH to yield compound **1**. The fraction eluted with H_2O-EtOH (60–40) was similarly treated to yield compounds **2** and **3**.

Compound **1**: 3′,4′-dihydroxy-β-phenyl ethyl caffeate-4′-β-*O*-D-galactopyranosyl-(1‴→4″)-α-*O*-L-rhamnopyranoside [= 9′-decarboxyrosmarinicacid-4′-*O*(1→4) galactosyl rhamnoside; Teucrol-4′-*O*-(1→4) galactosylrhamnoside]; brownish-yellow amorphous powder (30 mg), R_f value: 0.50 (H_2O), 0.57 (HOAc), 0.53 (BAW), blue fluorescence in UV, canary yellow with NH_4OH and intense green with $FeCl_3$; UV: λ_{max} (nm) in MeOH ($250_{shoulder}$, 290, 330). MS: electron-spray ionization in negative mode $(ESI)^-$ *m/z*: 624 (M+), 623 (M-H); at low cone voltage (35 v), a doubly charged ion $[M-2H]^{2-}$ at *m/z* 311; at higher cone voltage (70 v), *m/z* 461 and *m/z* 161 (loss of hexose unit); ESI^+, the most abundant ion is *m/z* 647 $[M + Na]^+$; at a cone voltage of 70 v, fragments with *m/z* 485 and 163.

1D-^{1}H and ^{1}H-^{1}HCOSY NMR: 9′-decarboxyrosmarinic acid moiety: δ ppm 2.66 (t, *J* = 7 Hz, H-7), 3.85 (hidden by sugar proton resonances), 6.45 (dd, *J* = 8 Hz and 2 Hz, H-6′), 6.62 (d, *J* = 8 Hz, H-5′), 6.65 (d, *J* = 2 Hz, H-2′), 6.15 (d, *J* = 16 Hz, H-α), 6.7 (d, *J* = 7.5 Hz, H-5), 6.86 (dd, *J* = 7.5 Hz and 2 Hz, H-6), 7.02 (d, *J* = 2 Hz, H-2), 7.45 (d, *J* = 16 Hz, H-β); *O*-galactosyl rhamnoside moiety: δ ppm: 5.06 (d, *J* = 2 Hz, rhamnoside H-1‴, 4.88 (d, *J* = 8.5 Hz, β-galactopyranosyl H-1″, 3.15–3.90 (m, 8′ methylenic and sugar protons overlapped with exchangable proton resonance), 0.95 (d, *J* = 6 Hz, rhamnose-CH_3).

^{13}C NMR: 9′-decarboxy rosmarinic acid: δ ppm 38.5 (C-7′), 66.6 (C-8′), 129.3–131.7 (C-1′), 116.5 (C-2′), 145.1 (C-3′), 143.6–142.7 (C-4′), 115.6 (C-5′), 119.7 (C-6′), 145.73 (C-7), 114.8 (C-8), 165.8 (C-9), 125.6 (C-1), 113.7 (C-2), 145.1 (C-3), 148.6 (C-4), 115.9 (C-5), 121.7 (C-6); galactosyl moiety: δ ppm 101.4 (C-1‴), 70.7 (C-2‴), 70.5 (C-3‴), 79.3 (C-4‴), 70.5 (C-5‴); rhamnosyl moiety: 19.4 (C-Me), 112.4 (C-1″), 71.8 (C-2″), 74.7 (C-3″), 68.9 (C-4″), 74.7 (C-5″), 69.3 (C-6″).

Acid hydrolysis of 1

Normal acid hydrolysis (2 N aqueous HCl, 3 h, 100 °C) gave caffeic acid and 3,4-dihydroxy-β-phenyl ethanol (CoPC) in the ethyl acetate extract of the hydrolysate and galactose as well as rhamnose (CoPC) in the aqueous phase left after ethyl acetate extraction of the hydrolysate. Mild acid hydrolysis (0.1 N HCl, 30 min, at 100 °C), followed by PPC of the ethyl acetate extract of the hydrolysate on Whatman 3 MM sheets, using BAW as solvent system, and inspection under UV light, afforded 9′-decarboxyrosmarinic acid in good agreement with previously reported data (El-Mousallamy et al., 2000).

Enzymatic hydrolysis of 1

Hydrolysis of **1** with β-galactosidase (BDH) at pH 5.0 (acetate buffer) for 48 h at 37 °C followed by CoPC of the product in solvent systems 1, 2, 3 and 4 yielded galactose as the only sugar.

Results and discussion

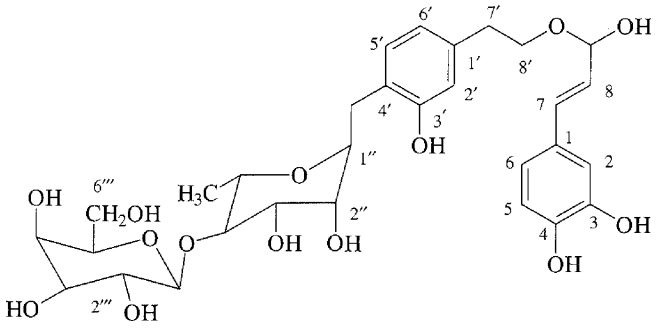
The hydroalcoholic extract of the aerial parts of *Blepharis ciliaris* collected in Egypt yielded compounds **1–3** applying a combination of polyamide and Sephadex LH-20 column chromatography. Data of compounds **2** and **3** were in full agreement with those reported for apigenin 7-*O*-glucoside (Markham et al., 1977; Nawwar et al., 1989) and apigenin 7-*O*-(3‴-acetyl-6″-*E*-*p*-coumaroyl glucoside) (Harraz et al., 1996), respectively. UV spectral maxima of compound **1** in MeOH ($250_{shoulder}$, 290, 330) indicated a caffeate ester (Harborne, 1994).

Normal acid hydrolysis of **1** afforded caffeic acid (CoPC, UV, ^{1}H and ^{13}C NMR) as well as 3,4-dihydroxy-β-phenyl-ethanol (chromatographic properties, UV, EI-MS and ^{1}H NMR) as the only phenolic components, together with L-rhamnose and D-galactose (CoPC). Compound **1** did not

resist the effect of the enzyme β-galactosidase. On the other hand, mild acid hydrolysis yielded decarboxyrosmarinic acid whose data agreed well with those previously reported (El-Mousallamy et al., 2000). It was, thus, concluded that compound **1** is a galactosylrhamnoside of decarboxyrosmarinic acid. The site of attachment of the sugar moiety to the phenolic one was demonstrated by the ^{1}H-NMR spectrum (DMSO-d_6) which revealed two distinct patterns of proton resonances. The first pattern is typical for decarboxyrosmarinic acid and comprises well-separated signals, except those of the 8′-methylenic protons which were determined by ^{1}H-^{1}H cosy experiment to be located at δ ppm 3.85, but overlapped with other sugar proton signals. The second pattern is characteristic for sugar protons and was found to contain one distinct α-$^1C_4$-rhamnose anomeric proton resonance at δ ppm 4.28 as a doublet of $J = 2$ Hz, together with one β-$^4C_1$ galactose anomeric proton resonance at δ ppm 4.78 (d, $J = 8.5$ Hz), and one rhamnose methyl protons doublet ($J = 6$ Hz) at δ ppm 0.95. Other sugar proton resonances appeared at δ ppm 3.30–3.85. The relatively upfield position of the anomeric proton of the galactose moiety indicated its connection to the other sugar through an alcoholic rather than to a phenolic hydroxyl. Connection through the latter would have caused a downfield shift. Similar chemical shifts are known in association with flavonoid glycosides (Harborne, 1994). These data together with the results of enzymatic hydrolysis, suggest the linkage of the two sugar molecules as galactosylrhamnoside to the phenolic hydroxyl group, resulting in a decarboxy rosmarinic acid galactosyl rhamnoside structure.

^{13}C NMR spectroscopy, including off-resonance and ^{1}H-^{13}C COSY, allowed the full assignment of all carbon resonances and identification of **1** as a decarboxyrosmarinic acid derivative bearing a 4′-*O*-substituent. This followed immediately from the direct comparison with the ^{13}C-NMR spectrum of the aglycone. In the aromatic region, both spectra exhibited close similarity. However, a distinction could be made, because of the upfield shift ($\Delta\delta = 1.7$ ppm) of the C-4′ carbon resonance of the 3′,4′-dihydroxy-β-phenyl ethyl moiety of **1** and the accompanying downfield shifts of the resonances of the *ortho* carbons, C-3′ and C-5′ ($\Delta\delta = 1.0$ and 1.1 ppm, respectively) and of the resonance of the *para*-carbon, C-6′ ($\Delta\delta = 1.7$ ppm) as well. Similar shifts have been observed in the ^{13}C-NMR of phenolic glycosides (Nawwar et al., 1982). The number and characteristic shifts of the ^{13}C sugar signals indicated the presence of one rhamnose and one galactose, all existing in the pyranose form. The presence of one rhamnose moiety was substantiated by the sp^3 methyl resonance at δ ppm 18.2. The position of the methyl signal indicated the attachment of the primary rhamnose hydroxyl to the 4′-phenolic hydroxyl of the aglycone, since attachment with other rhamnose hydroxyls would have caused a downfield shift of the methyl to *ca.* 21 ppm (Markham et al., 1978).

The α-configuration of the rhamnose moiety followed from its C-1 signal at 101.4 ppm, while the β-configuration of the galactose moiety was concluded from the signal of its anomeric carbon at 102.4 ppm. Chemical shifts of both sugars indicated the linkage of C-1 of galactose to C-4 of rhamnose, whose resonance was down-field shifted to δ ppm 79.0 in comparison with free rhamnose (Breitmaier & Voelker, 1978). An accompanying γ-upfield shift caused by the galactosyl C-1 was also detected for the resonances of C-3 and C-5 (δ ppm 70.5 and 70.5) of the rhamnosyl moiety, thus confirming the structure of the sugar case in **1** to be β-*O*-D-galactopyranosyl (1‴→4″)-α-*O*-L-rhamnopyranoside, which represents a new natural dioside. Consequently, compound **1** was identified as 3′,4′-dihydroxy-β-phenyl ethyl caffeate-4′-β-*O*-D-galactopyranosyl-(1‴→4″)-β-*O*-L-rhamnopyranoside, a new phenolic dioside which has not been reported before in nature. Rosmarinic acid and its derivatives isolated from different sources exhibited significant biological activities comprising: anti-inflammatory (Gracza et al., 1985), anti-HIV (Arda et al., 1997), antiviral (Borkowski et al., 1996), antithrombic and antiplatelet (Zou et al., 1993) and immunstimulatory (Sawicka et al., 1994) activities. Accordingly, the different biological activities exhibited by the hydroalcoholic extract of *B. ciliaris* under investigation (Zakaria, 2001) can possibly be attributed to compound **1**, being a rosmarinic acid derivative. Further studies are in progress to investigate the pharmacotoxicity and pharmacodynamics of this compound.



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